

Amendments to the Claims:

Please amend claims 5-7, 10, 13, 20, 33, 36, 39, 42-44, 47-52, as described below. Also, please add new claims 54-55, as described below. In addition, attached to the end of this Preliminary Amendment is a marked-up version of the amended claims, marked to show all of the changes relative to the previous version.

1. A method for detecting a mutation in fungal nucleic acid wherein the presence of said mutation gives rise to fungal resistance to a strobilurin analogue or any other compound in the same cross resistance group said method comprising identifying the presence or absence of said mutation in fungal nucleic acid using any (or a) single nucleotide polymorphism detection technique.
2. A method for detecting a mutation in fungal nucleic acid wherein the presence of said mutation gives rise to fungal resistance to a strobilurin analogue or any other compound in the same cross resistance group said method comprising detecting the presence of an amplicon generated during a PCR reaction wherein said PCR reaction comprises contacting a test sample comprising fungal nucleic acid with a diagnostic primer in the presence of appropriate nucleotide triphosphates and an agent for polymerisation wherein the detection of said amplicon is directly related to the presence or absence of said mutation in said nucleic acid.
3. A method for detecting a mutation in fungal nucleic acid wherein the presence of said mutation gives rise to fungal resistance to a strobilurin analogue or any other compound in the same cross resistance group which method comprises contacting a test sample comprising fungal nucleic acid with an appropriate diagnostic primer in the presence of appropriate nucleotide triphosphates and an agent for polymerisation, such that the diagnostic primer is

extended either when the said mutation is present in the sample or when wild type sequence is present; and detecting the presence or absence of the said mutation by reference to the presence or absence of a diagnostic primer extension product.

4. A method for detecting a mutation in fungal nucleic acid according to claim 2 wherein said method comprises contacting a test sample comprising fungal nucleic acid with a diagnostic primer for the specific mutation in the presence of appropriate nucleotide triphosphates and an agent for polymerisation, such that the diagnostic primer is extended when the said mutation is present in the sample; and detecting the presence or absence of the said mutation by reference to the presence or absence of a diagnostic primer extension product.

5. A method according to claim 1 wherein the mutation is present in a fungal cytochrome *b* gene where said mutation results in the inhibition of a strobilurin analogue or any other compound in the same cross resistance group to the active site of the cytochrome *b* protein but still allows the respiration process to occur.

6. A method according to claim 5 wherein the mutation in the fungal nucleic acid results in the replacement of a glycine residue at the position corresponding to *S. cerevisiae* cytochrome *b* residue 143 in the encoded protein with an amino acid selected from the group arginine, serine, cysteine, valine, aspartic acid and alanine.

7. A method according to claim 1 wherein the mutation in the fungal nucleic acid results in the replacement of a glycine residue at the position corresponding to *S. cerevisiae* cytochrome *b* residue 143 in the encoded protein with an alanine.

8. A method according to claim 2 for the detection of a mutation in a fungal cytochrome *b* gene resulting in a G₁₄₃A replacement in the encoded protein wherein said mutation gives rise to fungal resistance to a strobilurin analogue or any other compound in the same cross resistance group said method comprising detecting the presence of an amplicon generated during a PCR reaction wherein said PCR reaction comprises contacting a test sample comprising fungal nucleic acid with a primer in the presence of appropriate nucleotide triphosphates and an agent for polymerisation wherein the detection of said amplicon is directly related to presence or absence of said mutation in said nucleic acid.

9. A method according to claim 3 for the detection of a mutation in a fungal cytochrome *b* gene resulting in a G₁₄₃A replacement in the encoded protein said method comprising contacting a test sample comprising fungal nucleic acid with a diagnostic primer for the mutation resulting in a G₁₄₃A replacement in the encoded protein in the presence of appropriate nucleotide triphosphates and an agent for polymerisation, such that the diagnostic primer is extended when a mutation is present in the sample resulting in a G₁₄₃A replacement in the encoded protein; and detecting the presence or absence of the said mutation by reference to the presence or absence of a diagnostic primer extension product.

10. A method according to claim 1 wherein the fungal gene is present in a plant pathogenic fungus selected from the group consisting of *Plasmopara viticola*, *Erysiphe graminis* f.sp. *tritici/hordei*, *Rhynchosporium secalis*, *Pyrenophora teres*, *Mycosphaerella graminicola*, *Venturia inaequalis*, *Mycosphaerella fijiensis* var. *difformis*, *Sphaerotheca fuliginea*, *Uniclinula necator*, *Colletotrichum graminicola*, *Pythium aphanidermatum*, *Collectotrichum gloeosporioides*, *Oidium lycopersicum*, *Magnaporthe grisea*, *Phytophthora infestans*, *Leveillula taurica*, *Pseudoperonospora cubensis*, *Alternaria solani*, *Rhizoctonia solani*, *Mycosphaerella musicola* and *Cercospora arachidola*.

11. A method for detecting fungal resistance to a strobilurin analogue or any other compound in the same cross resistance group said method comprising identifying the presence or absence of a mutation in fungal nucleic acid wherein the presence of said mutation gives rise to resistance to a strobilurin analogue or any other compound in the same cross resistance group said method comprising identifying the presence or absence of a single nucleotide polymorphism occurring at a position corresponding to one or more of the bases in the triplet coding for the amino acid at the position corresponding to *S. cerevisiae* cytochrome *b* residue 143 in the cytochrome *b* protein.

12. A method according to claim 11 wherein the said single nucleotide polymorphism occurs at a position corresponding to the second base in the triplet coding for the amino acid at the position corresponding to *S. cerevisiae* cytochrome *b* residue 143.

13. A method according to claim 1 wherein the single nucleotide polymorphism which is detected is a G to C base change occurring at a position corresponding to the second base in the triplet coding for the amino acid at the position corresponding to *S. cerevisiae* cytochrome *b* residue 143 in the cytochrome *b* protein.

14. A fungal DNA sequence encoding all or part of a cytochrome *b* protein wherein said DNA sequence encodes a glycine residue at the position corresponding to *S. cerevisiae* cytochrome *b* residue 143 and is obtainable from a fungus selected from the group consisting of *Plasmopara viticola*, *Erysiphe graminis* f.sp. *tritici/hordei*, *Rhynchosporium secalis*, *Pyrenophora teres*, *Mycosphaerella graminicola*, *Venturia inaequalis*, *Mycosphaerella fijiensis* var. *difformis*, *Sphaerotheca fuliginea*, *Ucinula nectar*, *Colletotrichum graminicola*, *Pythium aphanidermatum*, *Collectotrichum gloeosporioides*, *Oidium lycopersicum*, *Magnaporthe grisea*, *Phytophthora infestans*, *Leveillula taurica*, *Psuedoperonospora cubensis*, *Alternaria solani*, *Rhizoctonia solani*, *Mycosphaerella musicola* and *Cercospora arachidola*.

15. A fungal DNA sequence according to claim 14 comprising all or part of a DNA sequence selected from the group SEQ ID NO 1, SEQ ID NO 2, SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 11, SEQ ID NO 12, SEQ ID NO 13, SEQ ID NO 14, SEQ ID NO 15, SEQ ID NO 16, SEQ ID NO 17, SEQ ID NO 18, SEQ ID NO 19, SEQ ID NO 20, SEQ ID NO 21

16. A fungal DNA sequence encoding all or part of a cytochrome *b* protein which, when said sequence is lined up against the corresponding wild type DNA sequence encoding a cytochrome *b* protein, is seen to contain a single nucleotide polymorphism mutation at a position in the DNA corresponding to one or more of the bases in the triplet coding for the amino acid at the position corresponding to *S. cerevisiae* cytochrome *b* residue 143 in the protein which results in the replacement of the normal glycine residue with an alternative amino acid with the proviso that said DNA sequence is not the *Mycena galopoda* sequence encoding cytochrome *b*.

17. A fungal DNA sequence according to claim 16 wherein said single nucleotide polymorphism mutation occurs at a position corresponding to the second base in the triplet coding for the amino acid at the position corresponding to *S. cerevisiae* cytochrome *b* residue 143 in the protein which results in the replacement of the normal glycine residue with an alternative amino acid with the proviso that said DNA sequence is not the *Mycena galopoda* sequence encoding cytochrome *b*.

18. A fungal DNA sequence encoding all or part of a cytochrome *b* protein wherein said DNA sequence encodes an alanine at the position corresponding to *S. cerevisiae* cytochrome *b* residue 143 and is obtainable from a fungus selected from the group consisting of *Plasmopara viticola*, *Erysiphe graminis* f.sp. *tritici/hordei*, *Rhynchosporium secalis*, *Pyrenophora teres*, *Mycosphaerella graminicola*, *Venturia inaequalis*, *Mycosphaerella fijiensis* var. *difformis*, *Sphaerotheca*

fuliginea, *Unicinula nectar*, *Colletotrichum graminicola*, *Pythium aphanidermatum*, *Collectotrichum gloeosporioides*, *Oidium lycopersicum*, *Magnaporthe grisea*, *Phytophthora infestans*, *Leveillula taurica*, *Psuedoperonospora cubensis*, *Alternaria solani*, *Rhizoctonia solani*, *Mycosphaerella musicola* and *Cercospora arachidola*.

19. A fungal DNA sequence encoding all or part of a cytochrome *b* protein according to claim 18 wherein said DNA sequence contains a single nucleotide polymorphism which results in the replacement of the normal guanine residue with a cytosine residue at a position corresponding to the second base in the triplet coding for the amino acid at the position corresponding to *S. cerevisiae* cytochrome *b* residue 143 in the protein with the proviso that said DNA sequence is not the *Mycena galopoda* sequence encoding cytochrome *b*.

20. A fungal DNA sequence according to claim 16 comprising all or part of a sequence selected from the group SEQ ID NO 176, SEQ ID NO 177, SEQ ID NO 178, SEQ ID NO 179, SEQ ID NO 180, SEQ ID NO 181, SEQ ID NO 182, SEQ ID NO 183, SEQ ID NO 184, SEQ ID NO 185, SEQ ID NO 186, SEQ ID NO 187, SEQ ID NO 188, SEQ ID NO 189, SEQ ID NO 190, SEQ ID NO 191, SEQ ID NO 192, SEQ ID NO 193, SEQ ID NO 194, SEQ ID NO 195, SEQ ID NO 196.

21. A fungal cytochrome *b* protein which confers fungal resistance to a strobilurin analogue or a compound within the same cross resistance group wherein in said protein a normal glycine residue is altered due to the presence of a mutation in the DNA coding for said protein said mutation occurring at a position corresponding to one or more of the bases in the triplet coding for the amino acid at the position corresponding to *S. cerevisiae* cytochrome *b* residue 143 in the protein with the proviso that protein is not the *Mycena galopoda* cytochrome *b* protein.

22. A fungal cytochrome *b* protein according to claim 21 wherein in said protein a normal glycine residue is altered due to the presence of a mutation

in the DNA coding for said protein said mutation occurring at a position corresponding to the second base in the triplet coding for the amino acid at the position corresponding to *S. cerevisiae* cytochrome *b* residue 143 in the protein with the proviso that protein is not the *Mycena galopoda* cytochrome *b* protein.

23. A method for the detection of a mutation in fungal cytochrome *b* gene resulting in the replacement of a glycine residue in the encoded protein at the position corresponding to *S. cerevisiae* cytochrome *b* residue 143 said method comprising identifying the presence and absence of said mutation in a sample of fungal nucleic acid wherein any (or a) single nucleotide polymorphism detection method is based on the sequence information from around 30 to 90 nucleotides upstream and/or downstream of the position corresponding to one or more of the bases in the triplet coding for the amino acid at the position corresponding to *S. cerevisiae* cytochrome *b* residue 143 in either the wild type or mutant protein.

24. A method according to claim 23 wherein any (or a) single nucleotide polymorphism detection method is based on the sequence information from around 30 to 90 nucleotides upstream and/or downstream of the position corresponding to the second base in the triplet coding for the amino acid at the position corresponding to *S. cerevisiae* cytochrome *b* residue 143 in either the wild type or mutant protein.

25. An allele specific oligonucleotide capable of binding to a fungal nucleic acid sequence encoding a wild type cytochrome *b* protein selected from the group consisting of *Plasmopara viticola*, *Erysiphe graminis f.sp. tritici/hordei*, *Rhynchosporium secalis*, *Pyrenophora teres*, *Mycosphaerella graminicola*, *Venturia inaequalis*, *Mycosphaerella fijiensis* var. *difformis*, *Sphaerotheca fuliginea*, *Unicinula nectar*, *Colletotrichum graminicola*, *Pythium aphanidermatum*, *Collectotrichum gloeosporioides*, *Oidium lycopersicum*, *Magnaporthe grisea*, *Phytophthora infestans*, *Leveillula taurica*, *Psuedoperonospora cubensis*, *Alternaria solani*, *Rhizoctonia solani*, *Mycosphaerella musicola* and *Cercospora arachidola* wherein said oligonucleotide

comprises a sequence which recognises a nucleic acid sequence encoding a glycine residue at the position corresponding to *S. cerevisiae* cytochrome *b* residue 143.

26. An allele specific oligonucleotide capable of binding to a fungal nucleic acid sequence encoding a mutant cytochrome *b* protein wherein said oligonucleotide comprises a sequence which recognises a nucleic acid sequence encoding an amino acid selected from the group arginine, serine, cysteine, valine, aspartic acid, glutamic acid, tryptophan, or alanine at the position corresponding to *S. cerevisiae* cytochrome *b* residue 143.

27. A diagnostic primer or a diagnostic oligonucleotide capable of binding to a template comprising a mutant type fungal cytochrome *b* nucleotide sequence wherein the final 3' nucleotide of the primer or oligonucleotide corresponds to a nucleotide present in said mutant form of a fungal cytochrome *b* gene and the presence of said nucleotide gives rise to fungal resistance to a strobilurin analogue or any other compound in the same cross resistance group.

28. A diagnostic primer according to claim 27 wherein either the penultimate nucleotides (-2) or (-3) of the primer is not the same as that present in the corresponding position in the wild type cytochrome *b* sequence.

29. One or more diagnostic primers for detecting a G₁₄₃A mutation in a fungal cytochrome *b* gene selected from the group consisting of SEQ ID NO 22, SEQ ID NO 23, SEQ ID NO 24, SEQ ID NO 25, SEQ ID NO 26, SEQ ID NO 27, SEQ ID NO 28, SEQ ID NO 29, SEQ ID NO 30, SEQ ID NO 31, SEQ ID NO 32, SEQ ID NO 33, SEQ ID NO 34, SEQ ID NO 35, SEQ ID NO 36, SEQ ID NO 37, SEQ ID NO 38, SEQ ID NO 39, SEQ ID NO 40, SEQ ID NO 41, SEQ ID NO 42 a derivatives thereof wherein the final nucleotide at the 3' end is identical to the sequences given above and wherein up to 10, such as up to 8, 6, 4, 2, 1, of the

remaining nucleotides may be varied without significantly affecting the properties of the diagnostic primer.

30. An allele specific oligonucleotide probe capable of detecting a fungal cytochrome *b* gene polymorphism at a position in the DNA corresponding to one or more of the bases in the triplet coding for the amino acid at the position corresponding to *S. cerevisiae* cytochrome *b* residue 143 in the protein.

31. An allele specific oligonucleotide probe according to claim 30 wherein said polymorphism is at a position in the DNA corresponding to the second base in the triplet coding for the amino acid at the position corresponding to *S. cerevisiae* cytochrome *b* residue 143 in the protein.

32. An allele specific oligonucleotide probe according to claim 31 wherein the polymorphism is a guanine to cytosine base change.

33. A diagnostic kit comprising one or more of the diagnostic primers as claimed in claim 27, nucleotide triphosphates, polymerase, and buffer solution.

34. A method of detecting plant pathogenic fungal resistance to a fungicide said method comprising detecting a mutation in a fungal gene wherein the presence of said mutation gives rise to resistance to a fungicide whose target protein is encoded by a mitochondrial gene using any (or a) single nucleotide polymorphism technique.

35. A method of detecting plant pathogenic fungal resistance to a fungicide said method comprising detecting the presence of an amplicon generated during a PCR reaction wherein said PCR reaction comprises contacting a test sample comprising fungal nucleic acid with a primer in the presence of appropriate nucleotide triphosphates and an agent for

polymerisation wherein the detection of said amplicon is directly related to presence or absence of a mutation in said nucleic acid wherein the presence of said mutation gives rise to resistance to a fungicide whose target protein is encoded by a mitochondrial gene.

36. A method of detecting plant pathogenic fungal resistance to a fungicide to claim 34 said method comprising contacting a test sample comprising fungal nucleic acid with a diagnostic primer for a specific mutation the presence of which gives rise to fungicide resistance in the presence of appropriate nucleotide triphosphates and an agent for polymerisation, such that the diagnostic primer is extended when the said mutation is present in the sample; and detecting the presence or absence of the said mutation by reference to the presence or absence of a diagnostic primer extension product.

37. A method of detecting and quantifying the frequency of a mutation giving rise to plant pathogenic fungal resistance to a fungicide whose target protein is encoded by a mitochondrial gene said method comprising detecting the presence or absence of a mutation in a fungal gene wherein the presence of said mutation gives rise to fungal resistance to a strobilurin analogue or any other compound in the same cross resistance group said method comprising identifying and quantifying the presence or absence of said mutation in fungal nucleic acid using any (or a) single nucleotide polymorphism detection technique.

38. A method according to claim 37 said method comprising detecting the presence of an amplicon generated during a PCR reaction wherein said PCR reaction comprises contacting a test sample comprising fungal nucleic acid with appropriate primers in the presence of appropriate nucleotide triphosphates and an agent for polymerisation wherein the detection of said amplicon is directly related to both the presence and absence of a mutation in said nucleic acid wherein the presence of said mutation gives rise to resistance to a fungicide

whose target protein is encoded by a mitochondrial gene, and detecting and quantifying the relative presence and absence of the said mutation by reference to the presence or absence of an amplicon generated during the PCR reaction.

39. A method according to claim 37 comprising contacting a test sample comprising fungal nucleic acid with diagnostic primers to detect both the presence and absence of a specific mutation in said nucleic acid the presence of which gives rise to fungicide resistance to a fungicide whose target protein is encoded by a mitochondrial gene, in the presence of appropriate nucleotide triphosphates and an agent for polymerisation, such that the diagnostic primers relating to the absence and the presence of the specific mutation are extended when the appropriate fungal template is present in the sample; and detecting and quantifying the relative presence and absence of the said mutation by reference to the presence or absence of diagnostic primer extension products.

40. A method of selecting an active fungicide and optimal application levels thereof for application to a crop comprising analyzing a sample of a fungus capable of infecting said crop and detecting and/or quantifying the presence and/or absence of a mutation in a gene from said fungus wherein the presence of said mutation may give rise to resistance to a fungicide whose target protein is encoded by a mitochondrial gene and then selecting an active fungicide and optimal application levels thereof.

41. A method according to claim 40 wherein the detection method uses any (or a) single nucleotide polymorphism detection technique.

42. A method according to claim 40 wherein the detection method comprises contacting a test sample comprising fungal nucleic acid with a diagnostic primer for the specific mutation in the presence of appropriate nucleotide triphosphates and an agent for polymerisation, such that the diagnostic primer is extended when the said mutation is present in the sample;

and detecting the presence or absence of the said mutation by reference to the presence or absence of a diagnostic primer extension product.

43. A method of controlling fungal infection of a crop comprising applying a fungicide to the crop wherein said fungicide is selected according to claim 40.

44. A method according to claim 34 wherein the fungicide is a strobilurin analogue or any other compound in the same cross resistance group.

45. A method for detecting fungal resistance to a strobilurin analogue or any other compound in the same cross resistance group said method comprising identifying the presence or absence of a mutation in fungal nucleic acid wherein the presence of said mutation gives rise to resistance to a strobilurin analogue or any other compound in the same cross resistance group said method comprising identifying the presence or absence of a single nucleotide polymorphism occurring at a position in the DNA corresponding to one or more of the bases in the triplet coding for the amino acid at the position corresponding to *S. cerevisiae* cytochrome *b* residue 143 in the cytochrome *b* protein.

46. A method for detecting fungal resistance to a strobilurin analogue according to claim 45 said method comprising identifying the presence or absence of a single nucleotide polymorphism occurring at a position in the DNA corresponding to the second base in the triplet coding for the amino acid at the position corresponding to *S. cerevisiae* cytochrome *b* residue 143 in the cytochrome *b* protein.

47. A method according to claim 1 wherein the method of detection and/or quantifying is based on fluorescence detection of diagnostic primer extension products.

48. A method according to claim 1 wherein the method of detection involves the use of the Scorpion™ detection system.

49. A method according to claim 34 wherein said mutation occurs at a position in the DNA corresponding to the second base in the triplet coding for the amino acid at the position corresponding to *S. cerevisiae* cytochrome *b* residue 143 in the cytochrome *b* protein.

50. A method according to claim 34 wherein the said mutation is a guanine to cytosine change resulting in a G₁₄₃A replacement in the encoded protein where a wild type glycine residue is substituted with an alanine residue at the position corresponding to *S. cerevisiae* cytochrome *b* residue 143.

51. A computer readable medium having stored thereon any of the sequences in claim 1 including all or part of a DNA sequence or protein sequence encoding a mutant cytochrome *b* protein as herein described wherein the presence of a mutation gives rise to fungal resistance to a strobilurin analogue or any compound in the same cross resistance group; all or part of a DNA or protein sequence encoding a wild type cytochrome *b* sequence from a fungus selected from the group *Plasmopara viticola*, *Erysiphe graminis f.sp. tritici/hordei*, *Rhynchosporium secalis*, *Pyrenophora teres*, *Mycosphaerella graminicola*, *Venturia inaequalis*, *Mycosphaerella fijiensis* var. *difformis*, *Sphaerotheca fuliginea*, *Unicinula nectar*, *Colletotrichum graminicola*, *Pythium aphanidermatum*, *Collectotrichum gloeosporioides*, *Oidium lycopersicum*, *Magnaporthe grisea*, *Phytophthora infestans*, *Leveillula taurica*, *Psuedoperonospora cubensis*, *Alternaria solani*, *Rhizoctonia solani*, *Mycosphaerella musicola* and *Cercospora arachidola*; or any allele specific oligonucleotide; allele specific primer, allele specific oligonucleotide probe, common or diagnostic primer according to any of the preceding claims.

52. A diagnostic kit for use in a method according to claim 1.